

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 2 of 15

Attorney's Docket No.: 07148-
032001 / CGL99/0007US5A

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-9. (Canceled)

10. (Previously Presented) The nucleic acid fragment of claim 66, wherein said at least one mutation comprises a codon encoding His in place of the codon encoding Leu.

11-26. (Canceled)

27. (Previously Presented) An isolated nucleic acid comprising a full-length *Brassicaceae* delta-15 fatty acid desaturase coding sequence having at least one mutation in a region of said desaturase coding sequence encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said desaturase coding sequence non-functional and wherein said sequence includes said at least one mutation.

28. (Canceled)

29. (Previously Presented) The nucleic acid fragment of claim 27, wherein said sequence encodes a microsomal gene product.

30. (Canceled)

31. (Previously Presented) The nucleic acid fragment of claim 27, wherein said at least one mutation introduces a non-conservative amino acid substitution in said region.

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 3 of 15

Attorney's Docket No.: 07148-
032001 / CGL99/0007US5A

32. (Previously Presented) The nucleic acid fragment of claim 31, wherein the wild-type amino acid sequence of said motif comprises the sequence His-Asp-Cys-Gly-His (SEQ ID NO:9).

33. (Previously Presented) The nucleic acid fragment of claim 32, wherein said at least one mutation comprises a codon encoding Lys in place of the codon encoding Asp.

34. (Previously Presented) The nucleic acid fragment of claim 27, wherein said mutant desaturase coding sequence is from a *Brassica napus* plant.

35. (Previously Presented) A *Brassicaceae* plant containing a full-length coding sequence of a delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif and wherein said mutation renders the product of said desaturase gene non-functional.

36. (Canceled)

37. (Previously Presented) The plant of claim 35, wherein said mutation confers a decreased level of α -linolenic acid in seeds of said plant.

38. (Original) The plant of claim 35, wherein said mutant desaturase gene encodes a microsomal gene product.

39. (Original) The plant of claim 35, wherein said at least one mutation comprises a non-conservative amino acid substitution in said region.

40. (Previously Presented) The plant of claim 39, wherein the wild-type amino acid sequence of said motif comprises the sequence His-Asp-Cys-Gly-His (SEQ ID NO:9).

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 4 of 15

Attorney's Docket No.: 07148-
032001 / CGL99/0007US5A

41. (Previously Presented) The plant of claim 40, wherein said at least one mutation comprises a codon encoding Lys in place of the codon encoding Asp.

42. (Original) The plant of claim 35, wherein said mutant desaturase gene is from a *Brassica napus* plant.

43. (Original) The plant of claim 35, wherein said plant is a *Brassica napus* plant.

44. (Previously Presented) A *Brassicaceae* plant containing:

- a) a full-length coding sequence from a delta-12 fatty acid desaturase gene having at least one mutation, said at least one delta-12 gene mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif; and
- b) a full-length coding sequence from a delta-15 fatty acid desaturase gene having at least one mutation, said at least one delta-15 gene mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif;

wherein said delta-12 gene mutation and said delta-15 gene mutation render the products of said delta-12 desaturase gene and said delta-15 desaturase gene, respectively, non-functional.

45. (Previously Presented) The plant of claim 44, wherein said mutant genes confer a decreased level of α -linolenic acid in seeds of said plant compared to α -linolenic acid levels in corresponding seeds lacking said mutant genes.

46. (Previously Presented) A *Brassicaceae* or *Helianthus* plant containing a full-length coding sequence of a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a Tyr-Leu-Asn-Asn-Pro (SEQ ID NO:50) amino acid motif and wherein said mutation renders the product of said desaturase gene non-functional.

47-54. (Canceled)

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 5 of 15

Attorney's Docket No.: 07148-
032001 / CGL99/0007US5A

55. (Currently amended) A method for producing a *Brassicaceae* or *Helianthus* plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a *Brassicaceae* or *Helianthus* species;
- b) obtaining one or more plants from said cells;
- c) identifying at least one of said plants that contains a delta-12 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation in said delta-12 gene renders the product of said delta-12 desaturase gene non-functional; and
- d) producing said *Brassicaceae* or *Helianthus* plant line from said at least one plant, said *Brassicaceae* or *Helianthus* plant line having said at least one mutation in said delta-12 gene.

56. (Original) The method of claim 55, wherein said plant line yields an oil having a stabilized linoleic acid content from about 2.0 % to about 12.0 %.

57. (Currently amended) The method of claim 55, further comprising the steps of:

- e) inducing mutagenesis in cells of said *Brassicaceae* or *Helianthus* plant line;
- f) obtaining one or more plants from said cells of said *Brassicaceae* or *Helianthus* plant line;
- g) identifying at least one of said plants from step f) that contains a delta-15 fatty acid desaturase gene having at least one mutation, wherein said at least one mutation in said delta-15 gene is in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 desaturase gene non-functional; and

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 6 of 15

Attorney's Docket No.: 07148-
032001 / CGL99/0007US5A

h) producing a second Brassicaceae or Helianthus plant line from said at least one plant identified in step g), said second plant line having said at least one mutation in said delta-12 gene and said at least one mutation in said delta-15 gene.

58. (Original) The method of claim 55, wherein said starting variety is a *Brassica napus* variety.

59. (Original) The method of claim 58, wherein said mutation is in a first form of delta-12 fatty acid desaturase.

60. (Original) The method of claim 59, further comprising the step of crossing a plant of said plant line to a plant having a mutation in a second form of delta-12 fatty acid desaturase.

61. (Original) The method of claim 60, wherein said second mutation is in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif.

62. (Currently amended) The method of claim 59, further comprising the steps of:

- e) inducing mutagenesis in cells of said Brassicaceae or Helianthus plant line;
- f) obtaining one or more plants from said cells of said Brassicaceae or Helianthus plant line;
- g) identifying at least one of said plants from step f) that contains a second delta-12 fatty acid desaturase gene having at least one mutation, said second gene mutation in a region other than a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif; and

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 7 of 15

Attorney's Docket No.: 07148-
032001 / CGL99/0007US5A

- h) producing a second Brassicaceae or Helianthus plant line from said at least one plant identified in step g), said second Brassicaceae or Helianthus plant line having said first and second delta-12 gene mutations.

63. (Original) The method of claim 55, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.

64. (Currently amended) A method for producing a Brassicaceae plant line, comprising the steps of:

- a) inducing mutagenesis in cells of a starting variety of a Brassicaceae or ~~Helianthus~~ species;
- b) obtaining one or more plants from said cells;
- c) identifying at least one of said plants that contains a delta-15 fatty acid desaturase gene having at least one mutation, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said at least one mutation renders the product of said delta-15 desaturase gene non-functional; and
- d) producing said Brassicaceae plant line from said at least one plant, said Brassicaceae plant line having said mutation in said delta-15 gene.

65. (Original) The method of claim 64, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 8 of 15

Attorney's Docket No.: 07148-
032001 / CGL99/0007US5A

66. (Currently amended) An isolated nucleic acid comprising a **[[a]]** full length *Brassicaceae* or *Helianthus* delta-12 fatty acid desaturase coding sequence having at least one mutation in a region of said desaturase coding sequence encoding a Tyr-Leu-Asn-Asn-Pro (SEQ ID NO:50) amino acid motif, wherein said at least one mutation renders the product of said desaturase coding sequence non-functional and wherein said sequence includes said at least one mutation.

67. (Previously Presented) A method for identifying a mutation in a *Brassicaceae* plant, comprising:

- a) providing a *Brassicaceae* plant having a decreased α -linolenic acid content as compared with a corresponding control *Brassicaceae* plant; and
- b) identifying at least one mutation in a delta-15 fatty acid desaturase gene of said plant, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His amino acid motif, wherein said mutation renders the product of said delta-15 fatty acid desaturase gene non-functional.

68. (Previously Presented) The method of claim 67, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.

69. (Previously Presented) A method for identifying a mutation in a *Brassicaceae* or *Helianthus* plant, comprising:

- a) providing a *Brassicaceae* or *Helianthus* plant having an increased oleic acid content as compared with a corresponding control *Brassicaceae* or *Helianthus* plant; and
- b) identifying at least one mutation in a delta-12 fatty acid desaturase gene of said plant, said at least one mutation in a region encoding a His-Xaa-Xaa-Xaa-His

Applicant : Lorin R. DeBonte et al.
Serial No. : 08/572,027
Filed : December 14, 1995
Page : 9 of 15

Attorney's Docket No.: 07148-
032001 / CGL99/0007US\$A

amino acid motif, wherein said mutation renders the product of said delta-12 fatty acid desaturase gene non-functional.

70. (Previously Presented) The method of claim 69, wherein said identifying step comprises a technique selected from the group consisting of: PCR, 3SR and direct polynucleotide sequencing.